A NEW TYPE OF LETHAL HEREDITARY FACTOR IN DROSOPHILA

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY David Hiram Thompson

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A NEW TYPE OF LETHAL HEREDITARY FACTOR IN DROSOPHILA

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I INTRODUCTION

The question of the viability of females homozygous for sex-linked lethals was raised by Morgan and Bridges (1916). At that time they were unable to state definitely that a female with such a germinal constitution would not live but they made that assumption. A sex-linked lethal has been found in which the lethal bearing males live to transmit the lethal factor. This has made it possible to test the case and their assumption was found to be correct.

About two years ago there appeared in a cross between a wild female and a white ultra-bar several F_1 males which bore a new sex-linked character. In this character the wings are held erect over the back, the second pair of legs is functionless, and on the whole, these males resemble flies which have been over-etherized. When females heterozygous for this character are mated with erect males there appear in the next generation about equal numbers of erect sons, normal sons and normal-appearing females. But there are no erect females. Moreover none of these females have been found to be homozygous for the character and the sex ratio of two males to one female is always obtained in such a cross.

The sex ratio is of interest because it is the reverse of the sex ratio found in other sex-linked lethals. It also suggests the lethal (l_{sd}) described by Miss Stark, in which the lethal bearing males occasionally emerged and lived for a short time, although they were unable to use their legs for walking. The present lethal is similiar in that the lethal

bearing males emerge and the legs are often useless. However in this case the lethal bearing males live to propagate and if mated with females heterozygous for erect the lethal effect is obtained in one half of the females.

II METHOD OF CULTURE AND MAINTENANCE OF STOCK

The flies have been reared by the ordinary method and kept in a room with a controlled temperature of 24 C. When it is desired to examine the flies they are very lightly etherized and the erect flies quickly separated from the non-erects, after which they may be more heavily etherized. This precaution is taken in order to avoid confusion of erect-winged flies with over-etherized flies. This method of separation and classification of erect-winged flies is effective since no fly has been found with normal wings when under the influence of ether which later showed erect wings and conversely no fly classified as having erect wings has later shown the normal wing posture. In matings involving erect the offspring should be shaken out and classified each day if close approximations to expected ratios are desired, since any flies which may die ,due to crowding, cannot be classified. Classifications are made under the low power of a binocular microscope although they can be made equally well with the naked eye.

A stock pure for erect is not possible due to the total absence of females homozygous for erect. A stock of erect has been maintained by mating females heterozygous for erect with erect males in each generation. In such a mating one always gets one half erect sons and one half normal sons and a sex ratio of two males to one female. The stock has been examined

in each generation for any marked deviation in the one to two sex ratio and in the percentage of erect males. A close watch has been made for homozygous females.

III ORIGIN OF ERECT

On September 8, 1919, in a cross between a wild female and a white ultra bar male there were noticed several males crawling about the bottle with wings erect over the thorax as in over-etherized flies. This day's count of offspring consisted of 35 normal-winged females, 10 normal-winged males and 14 erect males. This female evidently was heterozygous for erect and since she was a virgin picked from a wild stock the character derived from her cannot be said to have arisen by hybridization of two stocks but from a pure wild stock. It may be of interest to note in connection with Miss Stark's work (1915) on lethals from freshly caught and inbred stocks that the wild stock in which this erect lethal arose had been in captivity for at least three or four years.

The erect males which must have been present in earlier counts of the original cross were overlooked probably because they were supposed to be over-etherized flies. The resemblence of these erect males to over-etherized flies is striking.

In the character "erect" it is the mesothorax which seems to be affected most because the wings are held erect over the back and the second pair of legs is usually doubled up beneath the body or else dragged along behind (figure 1). The wings stand at right angles with the main axis of the body and they usually are about parallel but occasionally touch or form an acute angle. The wings come into position as they expand and

remain so throughout the life of the fly, thus making flight impossible. No males have been found that transmit the factor for erect without having erect wings, and conversely no erect males have been found which do not transmit the characteristic. The second pair of legs usually are useless for walking, as they are either crossed and doubled up beneath the body or else they drag as the animal walks. Such flies have much difficulty in walking and occasionally become entangled in the food and die. This fact probably accounts partially for the erects appearing in somewhat fewer numbers than expected. Dead flies cannot be classified as to the erect character and are discarded. All figures given in this paper refer to counts of living flies. Flies with these feeble legs often partially recover the use of them for walking. In general flies cannot be classified by these feeble legs since they are rather irregular in appearance. They are part of the manifestation of the factor for erect, because erect flies with normal-appearing legs were selected for several generations, but the majority of the erect-winged males always had feeble legs. With moderate care in etherization erect is a readily recognized sex-linked character which is always definite in its behavior.

IV SEX RATIOS AND FREQUENCY OF ERECT

When females heterozygous for erect are out-crossed to non-erect males the usual 1:1 sex ratio is obtained and there is an approximation to equality of erect and normal males. The totals of such crosses are given in table 1. The scarcity of erect males here is partially accounted for by the number which died before they were counted, as the offspring were not removed daily in every case.

TABLE 1 Heterozygous female X non-erect male.

	Normal ♀♀	Erect ♂♂	Normal ♂♂
Total	1252	573	749
Percentage	48.6	22.3	29.1
Sex Ratio	1 : 1.06		

If females heterozygous for erect be mated to erect males the characteristic sex ratio of 1:2 is obtained and there are again nearly equal numbers of erect and normal males. The totals of these matings are given in table 2. It can be seen there that the results indicated by the totals are not the averages of widely varying ratios, for a close approximation to the expected ratio has been obtained in almost every mating.

TABLE 2 Heterozygous female X erect male.

Culture number	Erect ♀♀	Normal ♀♀	Erect ♂♂	Normal ♂♂
76.1	0	102	101	116
84.1	0	30	21	30
88.1	0	111	104	131
86.1	0	52	65	64
90.1	0	80	110	114
92.1	0	99	110	105
94.1	0	12	15	9
96.1	0	4	3	3
98.1	0	60	54	64
100.1	0	52	35	48
172.0	0	60	55	82
172.1	0	90	86	87
172.2	0	66	94	93
172.3	0	91	85	120
172.4	0	46	45	36
172.5	0	20	8	12
172.6	0	55	65	100
172.7	0	38	37	51
172.8	0	53	54	61
172.9	0	51	20	23
Total	0	1166	1167	1349
Percentage	0	31.7	31.7	36.6
Sex Ratio		1 : 2.16		

Unrelated non-erect females mated to erect males gave in F_1 equal numbers of normal-appearing females and males. Many of these F_1 females were then tested by unrelated non-erect males and all gave approximately equal numbers of erect and normal sons. This test brings out its characteristic sex-linked behavior.

Several thousand flies have been produced in crosses involving erect, and a constant watch has been made for homozygous erect females which would indicate crossing-over between a lethal factor and the erect factor, but no such females have been observed. Since the lethal effect and the character erect cannot be separated, it may be concluded that they are due to the same factor.

In the second generation from a cross between white bar females and erect males there appeared three females which carried their wings erect. These upon being tested proved to be heterozygous for erect but they transmitted this character of erect wings to part of their daughters. Considerable work has been done in testing these erect-winged females and it seems to indicate a dominant factor which makes erect dominant and is lethal in the homozygous condition if one erect factor is present. This new factor seems to be located in the autosomes, and unlike the erect factor it is quite irregular in its manifestation since certain environmental conditions seem to be necessary. This new dominating factor is similiar to erect in that it cannot be fixed, at least when the erect factor is present.

Homozygous erect females probably die in the egg or early larval stages since no approximation of 25% of pupae containing

dead flies has been found in crosses where they should occur, and no dead larvae noticed in good cultures.

V LOCATION OF ERECT FACTOR

In order to prove that this erect-winged lethal is a sex-linked character its linkage relations with bar and white have been obtained. White, bar, normal-winged females were crossed to red, full-eyed, erect males and the F_2 generation produced. The double-cross-over class was regarded as a cross-over in each region. The males of this cross show the linkage relations of erect with bar and white readily and are summarized in tables 3 and 4. There was found to be 36.9% of crossing-over between white and erect and 19.3% between bar and erect. These numbers added give the close approximation of 56.2% to the established 56% of crossing-over between white and bar. It seems evident that the factor for erect lies between white and bar somewhere near the locus 38.

TABLE 3 Bar-erect crossing-over

	Cross-over ♂♂	Non-cross-over ♂♂
Total	227	952
Percentage	19.3	

TABLE 4 White-erect crossing-over

	Cross-over ♂♂	Non-cross-over ♂♂
Total	238	407
Percentage	36.9	

VI DISCUSSION

An hereditary lethal may be defined as any heritable quality which prevents the individual from reaching the adult stage and reproducing. Lethal effects may also be thought of as including those qualities which lower the viability of any race of animals and may be termed racial lethals.

The first case of an hereditary lethal factor, which is quite typical, was demonstrated by Cuenot in mice. In mice there is a yellow coat color which cannot be fixed, that is, a line pure for yellow cannot be obtained. For instance, if a pure black be crossed to a yellow, one half of the offspring are yellow and one half are non-yellow and it is not evident which is the dominant character. But by further breeding experiments it is shown that yellow is dominant because, if the non-yellow offspring be mated together, none but non-yellows are obtained, showing that they are pure for the absence of yellow. If the yellow offspring be mated to the non-yellow equal numbers of the yellow and the non-yellow are again obtained, which shows that yellow is dominant, since it could have been present in the offspring only in the single dose.

Since yellow is dominant one would expect that if he mated yellow offspring together he would get yellows and non- yellows in the ratio of 3:1 and of the composition 1YY : 2Yy : 1yy, but on the contrary one gets a 2:1 ratio as was established by Little (1917) in large numbers of mice. Furthermore, one does not get pure yellow mice since large numbers have been tested and all have been found to be heterozygous for yellow. It was noticed that when the yellow by yellow cross was made the litters were

smaller than normal, being about three-fourths as large. It was then concluded that it was the pure yellow class of mice which was for some reason missing and that whenever two gametes united, each of which carried the yellow factor, the resulting zygote did not come to maturity.

Embryological work has recently been done on the yellow mice and it has been shown that when two yellow gametes unite they form a living embryo which lives an apparently normal existence up to the blastula stage, when the embryos become implanted in the uterine wall. At this point the two yellow factors manifest themselves, the embryo does not become attached but dies, probably from lack of nourishment. It has also been shown that the surviving yellow mice are mentally deficient. This is indicated by their behavoir in mazes where their standard of performamce is much lower than that of normal mice.

This yellow mouse case is a simple, clean cut, and rather completely understood example of how lethals act.

The great majority of known hereditary lethal factors are found in the fruit fly, Drosophila, and the largest number of these are sex-linked, that is, the factors are borne by the sex-chromosomes. This is because all known lethal factors are recessives, and sex-linked recessives are very easily found since one half the sons of a female heterozygous for any one of them are always pure for it. This follows since there are two sex-chromosomes in the female and one in the male which he always gets from his mother.

All lethal factors must be recessive in their lethal effect in order to be inherited because it is obvious that if they were

dominant there would be no way of transmitting them.

The number of dominant lethals arising by mutation is unknown but there are two cases known which are interesting in this connection. Muller (1921) has described a sex-linked lethal which is recessive under ordinary conditions of the germplasm but is a dominant lethal when a certain other allelomorph for this lethal factor is present. The other case is mentioned in this paper in which the erect factor seems to behave as a dominant lethal when two doses of the dominant accessory factor are present.

More is known of recessive than of dominant lethals. There is a variety of these which differ in the time of producing death, as well as the degree of viability. These variations seem to be well illustrated by the following series which begins with the most deadly of these lethals and grades down to their milder forms.

1. The first lethal to be considered is a sex-linked recessive factor reported by Miss Stark (1918). This causes the development of tumors in the male larvae and kills them before they pupate. Since this factor is carried by the sex-chromosomes it follows the rules for sex-linked inheritance. It can only be transmitted by the females, and the stock is maintained by mating heterozygous females to normal males in every generation. The sons of a heterozygous female should be equal in number to the daughters, but since one half of the sons die as larvae, one gets sons and daughters in the ratio of two daughters to one son, which is the usual ratio in sex-linked lethals.

When one mates females heterozygous for the lethal to normal males, all the sons that come to the mature stage are normal and cannot transmit the defect. The daughters are of two classes,

one half being heterozygous for the lethal factor and the other half being pure for its absence. As a result the former females will always transmit the defect to half their sons, while the latter can never transmit it. The principal characteristic of this lethal is that it kills one half of the males in the larval stage by the development of tumors.

2. The second case in this series was also described by Miss Stark (1915). This is similiar to the first in that one half of the sons of the heterozygous female were killed. However no evidence of a tumor was found. The males receiving the lethal factor lived through the larval stage and the early pupal stages, but died either before they emerged or shortly afterwards. Those that emerged could not walk, but fell over and lay on their sides, since the legs did not seem to move coordinately and were not strong enough to support the body. These flies died in a day or two and could not be used in breeding experiments. The points of interest in this lethal were that one half of the males were affected as in the previous case, but that the effect was delayed to the late pupal or young adult stages. It is also notable in connection with the following that the lethal bearing males which did emerge were very defective.

3. The next case in this series is the erect lethal described in this paper. The lethal factor is transmitted to one half the sons as in the two former cases, but does not have its complete effect in them since they go through the whole life cycle and emerge as adults. However, one can see the partial effect of the lethal because the wings are carried erect and resemble the the condition of rigor mortis. Also these flies have difficulty

in walking since the second pair of legs is almost always paralyzed and is dragged along as the animal walks. However these lethal bearing males live to breed which shows an advance over the last case. These sons which bear the lethal, when mated to females heterozygous for it should give the following types of offspring in equal numbers: daughters pure for the lethal, daughters heterozygous for the lethal, erect sons and normal sons, One does not get all these classes of flies because there are no females produced which are pure for the factor and therefore two males survive for every female. This shows that the females which get two doses die early in the life cycle. This stock is kept going by mating heterozygous females to erect males.

The outstanding points are that the factor does not have its complete effect in the lethal bearing males, but such an effect is obtained in the females homozygous for it. Here the ordinary lethal sex ratio is reversed being two males to one female instead of one male to two females.

4. For the next case in this series the character fused may be taken and regarded as a mild lethal which although not lethal to the individual is lethal to the race on account of the sterility of all homozygous females. Here the lethal bearing males live as in the erect case and show some lethal effects, in that the ocelli are missing and the wings are somewhat abnormal. These males when mated to heterozygous females give all the expected classes of flies, and in this respect are different from the previous case.

5. The factor for rudimentary wing behaves in the same way as fused except that the homozygous females occasionally lay a few eggs.

6. For the last case in the series any of the ordinary sex-linked factors may be mentioned such as bar eye, yellow body etc. In these instances there is no lethal effect causing certain whole classes of individuals to die nor is there complete sterility as in other cases but these stocks have a much lower viability than the normal wild type of fly.

This series shows the different points in the life cycle as well as the manner in which the lethals may manifest themselves.

In the evening primrose, Oenothera Lamarkiana, there are several phenomena which have puzzled those who attempt to explain heredity on the determinate basis. Among these peculiarities are the constant hybrids which apparently are races pure for certain characters although they are really heterozygous in composition. Another interesting fact is that when two apparently pure races are crossed there appear two and sometimes four classes of F_1 offspring. It has been shown by Muller (1917) that these cases can be paralleled in Drosophila by the proper use of lethals.

An example of constant hybrids in Drosophila is the dominant character "beaded wings" which under selection became a constant hybrid. The principal factor is a dominant which is lethal in the double dose just like the yellow mouse case. During selection a lethal factor appeared which was closely linked to the normal allelomorph for beaded. Thus the beaded stock consisted of flies heterozygous for both beaded and the lethal. If B represents the beaded factor and l the lethal factor and N their normal allelomorphs, there will be two types of eggs and two types of sperm with regard to these factors

namely $\underline{\quad NB}$ and $\underline{\quad lN\ }$. Neglecting crossing-over there are three possible combinations of these two types of eggs and sperm namely, NNBB, llNN, and lNBN. The first class dies as a result of the lethal action of two B's and the second as a result of the lethal action of the two l's while the third class lives. This class which lives is of the same composition as that of the parents and it repeats as a pure line except for occasional flies of other types produced by crossing-over between the lethal and beaded factors. If this apparently pure beaded stock be outcrossed to some other stock such as the wild fly there will be two classes of F_1 hybrids namely one half beaded flies without the lethal factor and one half normal flies heterozygous for the lethal. By crossing two different constant hybrid races there will be four classes of F_1 hybrids.

By making use of the idea of balanced lethals in connection with the erect stock one should be able to produce a stock in which all the females would be normal in appearance and the males all erect. This should be done by introducing an ordinary sex-linked lethal into the erect stock producing females of the composition $\frac{l}{l_e}$. Here the ordinary lethal is represented by l and the erect lethal by l_e. These females when mated to erect males would give erect sons only, the normal sons being killed by the introduced lethal. The daughters of this cross would be of the same composition as their mother and equal in number to the erect sons. The offspring that reach the adult stage are of the same composition as the parents and the process would repeat itself much as a pure line. Crossing-

over would give normal males and females not bearing the introduced lethal, the number of which would depend on the closeness of the lethal factor to the erect factor or the presence of other factors which may affect crossing-over. This balanced lethal should be of use in preserving stocks of other sex-linked lethals since it would give a higher percentage of flies bearing the lethal factor than other methods.

VII SUMMARY

1. A female from an inbred wild stock has been found to be heterozygous for a new sex-linked recessive character in which the wings are held erect and the legs are feeble.

2. This character follows the sex-linked method of transmission and shows linkage with bar and white eye.

3. The point of principal interest is that in mating females heterozygous for the factor with erect males the homozygous females which presumably would be erect do not appear and consequently there are twice as many males as females.

4. The lethal effect and the character erect seem to be manifestations of the same factor since it has not been possible to separate them in the several thousand flies which have been raised.

5. Erect males are so ill adapted to life under natural conditions that they may be regarded as lethal-bearing males which under laboratory conditions are permitted to live long enough to propagate. Females receiving two of these lethal factors die earlier in the life cycle. This indicates a greater effect of the two erect factors of the female as compared with

a single erect factor of the male.

6. The single erect factor of a female supposedly is without effect but it is of interest to note that a dominant accessory factor has appeared which makes the erect factor dominant so that these females have erect wings and occasionally feeble legs like the males.

7. There is some evidence which seems to indicate that two doses of this accessory factor also make the lethal manifestation of the erect factor dominant. This would explain the apparent scarcity of heterozygous females and erect males in tables 1 and 2.

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Figure 1. An erect male. (Sketched under camera lucida while lightly etherized.)